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Monitoring SPAAC with UV-VIS

General protocols from the dry lab

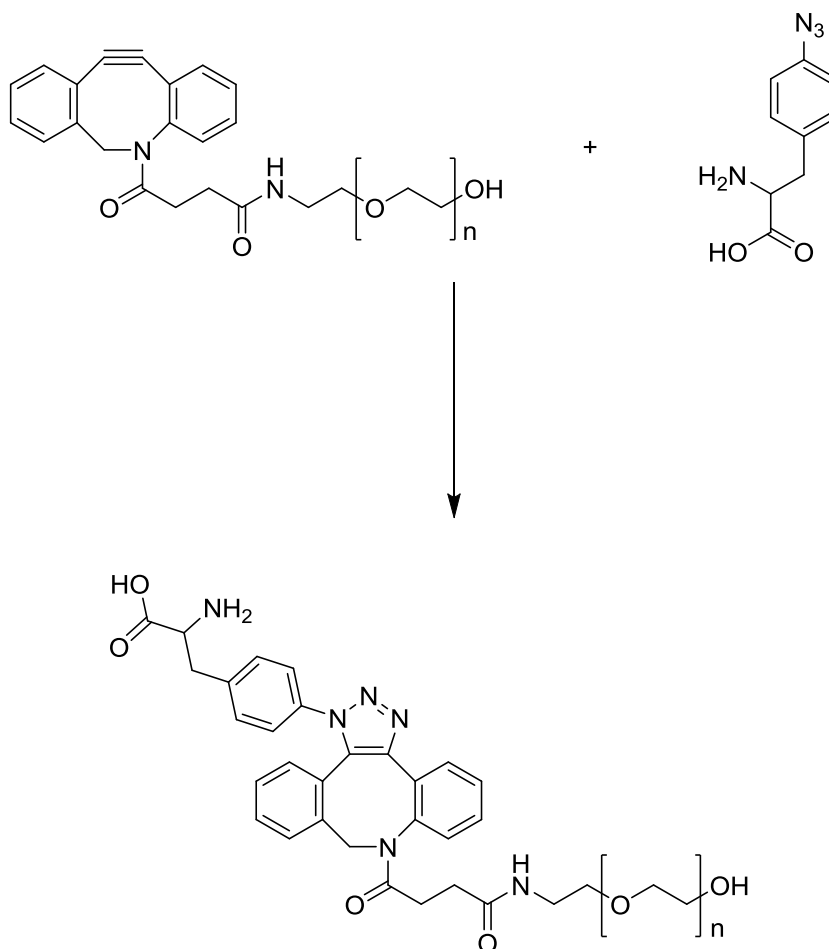


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1 Reagents

- Unnatural amino acid p-azido-L-phenylalanine (pAzF)
- DBCO-PEG (~10 kDa)
- Phosphate buffer (20 mM trisodium phosphate, pH 7.4)

2 Settings

Software: Spectramanager

Measurement type: Spectrum measurement and time-course measurement

Determination of cut-off value of the buffer with spectrum measurement		Determination of the calibration curve and determination of the characteristic triple bond peak with spectrum measurement		Time-course measurement	
Photometric mode	Abs	Photometric mode	Abs	Photometric mode	Abs
Response	Medium	Response	Medium	Response	Medium
UV/Vis bandwidth	1.0 nm	UV/Vis bandwidth	1.0 nm	UV/Vis bandwidth	2.0 nm
Scan Speed	40 nm/min	Scan Speed	40 nm/min	Wavelength	308 nm
Start	340 nm	Start	340 nm	Start	0 s
End	190 nm	End	220 nm	End	57600 s
Data interval	0.5 nm	Data interval	0.5 nm	Data interval	5.0 s
Vertical size	Auto	Vertical size	Auto	Vertical scale	Auto
Scan mode	Continuous	Scan mode	Continuous	Temperature	37 °C
Accumulation/cycle	Accumulation	Accumulation/cycle	Accumulation	Stirrer	250 rpm
No. Of cycles	2	No. Of cycles	2		
Temperature	20 °C	Temperature	37 °C		
		Stirrer	150 rpm		

3 Determination of cut-off value of the buffer

- Fill cuvettes with consecutively 1.0, 1.8, 2.0 and 3.0 mL acetone
- Determine which amount is sufficient to measure absorption

4 Determination of the calibration curve

- Prepare samples with subsequently 142.9, 100, 70, 79, 34.3, 24, 16.8 and 11.8 μM of DBCO-PEG in phosphate buffer
- Calculate the calibration curve to determine the concentration of DBCO in the reaction mixture using the Beer-Lambert Law:

$$E = -\log T = -\log \frac{I}{I_0} = \epsilon \cdot c \cdot l$$

E = extinction

T = transmission

I = intensity of the outgoing bundle

I_0 = intensity of the incoming bundle

ϵ = molar extinction coefficient

c = concentration (mol/L)

l = covered distance (cm)

5 Time course measurement

- Prepare a 47.6 μM pAzF stocksolution
- Prepare a 142.9 μM DBCO-PEG (~10 kDa) stocksolution
- Prepare blanc measurements by filling cuvettes with the same buffer used in the pAzF/DBCO-PEG stock solutions
- Prepare a sample containing 14.3 μM pAzF and 100 μM DBCO-PEG
- Measure according to the settings described above